MORPHOLOGICAL, CULTURAL AND PATHOGENIC VARIATIONS IN *SCLEROTIUM ROLFSII* SACC. CAUSING ROOT ROT OF SUGARBEET

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ABSTRACT - Variability in the *Sclerotium* root rot of sugarbeet (*Beta vulgaris* L.) pathogen (*Sclerotium rolfsii* Sacc.) was studied in 5 isolates collected from different sugarbeet-growing areas in Sriganganagar, Rajasthan State. Isolate Sr. 1 displayed significantly higher growth together with maximum number, size and weight of sclerotia than rest of the isolates. Oat meal agar and potato dextrose agar proved to be the best media for growth and formation of sclerotia, respectively. Isolate Sr. 1 proved most aggressive and caused higher incidence of seedling blight and root rot.

RÉSUMÉ - La variabilité de *Sclerotium rolfsii*, agent de la pourriture de racine de la betterave à sucre (*Beta vulgaris* L.), est étudiée pour 5 isolements provenant de différents champs de betterave à Sriganganagan, Etat du Rajasthan. L'isolat Sr. 1 montre une croissance significativement plus importante, ainsi qu'un nombre, une taille et un poids de sclérotes maximaux. Il est également le plus agressif. Le milieu PDA est le meilleur pour la formation des sclérotes tandis que le milieu gélosé à la farine d'avoine donne une meilleure croissance.

KEY WORDS : Sugarbeet, *Beta vulgaris*, root rot, *Sclerotium rolfsii*, variability.

Root rot of sugarbeet incited by *Sclerotium rolfsii* Sacc. is a very destructive disease in various countries including India. Since no disease management strategy can be perfect without understanding variability in pathogen, the present investigation was aimed to find out morphological, cultural, pathogenic and physiological variations in *S. rolfsii* causing root rot of sugarbeet in Rajasthan.

MATERIALS AND METHODS

Preparation of isolates: The fungus was isolated, purified and single-hyphal-tip cultures of 5 isolates collected from 5 distant areas of Sriganganagar designated as Sr. 1, Sr. 2, Sr. 3, Sr. 4, Sr. 5 were maintained on potato dextrose agar at 30 ± 1°C.

Source : MNHN, Paris

Cultural and morphological variations: Variation in growth and production of sclerotia (shape, size, colour and weight of sclerotia) were recorded by growing the isolates on 7 agar media viz., Asthana & Hawker's, Brown's, Czapek's, Oat Meal, Potato dextrose, Richards' and Sabouraud's medium. The amount of growth was recorded 4 days after inoculation by measuring radial growth along 2 diameters at right angle to each other from each of the 5 replicates. Number of sclerotia formed were counted 15 days after inoculation.

Morphology of sclerotia produced in culture: For each 15-day-old culture medium, 25 sclerotia were randomly picked up and observed visually for shape and colour. To record the size, each sclerotium was measured with the help of a calibrated microscope. To record weight, sclerotia of all the 4 replications were mixed (with a total of 100 sclerotia) and weighed on an analytical balance.

Morphology of sclerotia produced on host: Sugarbeet plants of cv. Ramonskaya raised under aseptic conditions in 30cm earthen pots were inoculated 4 months after sowing by placing 5 sclerotia (1.0-1.1mm dia) of each isolate in the root zone of each plant. Sclerotia were collected from 20-day-old culture grown in sterilized soil (100g) amended with 8% wheat bran. Eight plants were inoculated with each of the 5 isolates. At the time of harvesting (175-day-old crop), 4 roots infected with each isolate were selected. From each root, 25 sclerotia were randomly collected and their morphological observations were recorded.

Pathogenic variations: 2 types of symptom incited by *S. rolfsii* are seedling blight and root rot. Attempts were made to detect pathogenic variability amongst the isolates in both phases of the disease.

Seedling blight: Plants of sugarbeet cv. Ramonskaya were raised in 30cm earthern pots and inoculated in the manner as described above. Uninoculated plants served as check. The seedlings were accommodated in a green house where maximum and minimum temperatures fluctuated from 24 to 38°C and 10 to 26°C, respectively and relative humidity ranged from 21 to 94%. There were 5 replications with 3 pots (15 seedling in each pot) under each replication. The disease incidence was recorded 20 days after inoculation using the formula,

$$\text{Disease incidence} = \frac{\text{Number of infected plants}}{\text{Number of plants inoculated}} \times 100$$

Root rot: The plants of cv. Ramonskaya raised as described above were inoculated in the second week of February, 1982 when they were 4-month-old. The plants were accommodated in a green house were maximum and minimum temperatures fluctuated from 18 to 39°C and 6 to 26°C, respectively and relative humidity ranged from 21 to 97%. There were 6 replications with 10 pots in each replication. Observations of disease incidence were recorded at the time of harvest.

Source : MNHN, Paris

OBSERVATIONS

Cultural variation: Isolate Sr. 1 displayed significantly ($p = 0.05$) higher growth (Tab. 1) than rest of the isolates. The other 4 isolates did not differ significantly amongst themselves. All the media differred significantly ($p = 0.05$) from each other in favouring growth of the fungus except that the growth supported by Brown's agar, Sabouraud's agar and Czapek's agar was statistically at par ($p = 0.01$). Oat meal agar proved to be the best medium and Asthana & Hawker's agar the poorest. The isolates x media interactions were not significant.

Table 1: Radial growth (recorded 4 days after inoculation) of 5 isolates of *S. rolfsii* on different culture media at 30 ± 1°C. Data are mean of 5 replications.

Tableau 1: Croissance (4 jours après l'inoculation) des 5 isolats de *S. rolfsii* sur différents milieux à 30 ± 1°C (moyenne de 5 expériences).

	Radial growth (mm)					
Isolates / Medium	Sr. 1	Sr. 2	Sr. 3	Sr. 4	Sr. 5	Mean of media
P.D.A.	78.8	75.2	75.0	75.2	74.8	76.00
Oat Meal Agar	88.8	86.0	87.6	87.8	84.8	87.00
Brown's Agar	53.4	51.2	50.2	49.2	49.8	50.76
Richards' Agar	64.0	60.8	62.4	60.8	62.2	62.04
Sabouraud's Agar	50.0	51.4	51.6	51.6	50.2	50.96
Asthana & Hawker's Agar	45.8	43.4	48.2	47.8	41.2	45.28
Czapek's Agar	56.0	53.6	48.6	47.2	52.6	51.60
Mean of Isolate	62.4	60.23	60.51	60.08	59.37	

	C.D. at 5%	C.D. at 1%
Isolate (I)	1.77	N.S.
Media (M)	2.09	2.75
Interaction (I x M)	N.S.	N.S.

Maximum number of sclerotia were formed by isolates Sr. 1. This number was significantly ($p = 0.05$) more than that formed by any other isolate. There was no significant difference ($p = 0.05$) in number of sclerotia formed by Sr. 2, Sr. 3 and Sr. 4 (Tab. 2). Potato Dextrose Agar was most favourable medium for formation of sclerotia. The number of sclerotia formed on this medium was significantly ($p = 0.01$) more than that on any other medium. Richards' agar medium supported minimum number of sclerotia. Media x isolates interactions were not significant for number of sclerotia.

Source : MNHN, Paris

Table 2: Number of sclerotia (recorded 4 days after inoculation) formed by the 5 isolates of *S. rolfsii* on different culture media at 30 ± 1°C. Data are mean of 5 replications.

Tableau 2: Nombre de sclérotes formés (4 jours après l'inoculation) pour les 5 isolats de *S. rolfsii* sur différents milieux à 30 ± 1°C (moyenne de 5 expériences).

Number of sclerotia						
Isolates / Medium	Sr. 1	Sr. 2	Sr. 3	Sr. 4	Sr. 5	Mean of media
P.D.A.	173.6	167.4	156.2	153.4	143.2	158.76
Oat Meal Agar	150.8	145.6	142.8	153.2	137.4	145.96
Brown's Agar	60.4	50.0	49.8	48.2	57.6	55.20
Richards' Agar	36.6	38.0	35.2	35.6	36.6	36.40
Sabouraud's Agar	110.4	106.6	98.6	105.0	100.0	104.24
Asthana & Hawker's Agar	84.2	70.2	72.8	62.8	65.4	71.08
Czapek's Agar	52.8	54.0	52.8	57.8	53.8	54.24
Mean of Isolate	95.54	90.26	86.88	89.43	84.94	

	C.D. at 5%	C.D. at 1%
Isolate (I)	4.49	5.90
Media (M)	5.31	6.98
Interaction (I x M)	N.S.	N.S.

Morphological and physiological variations:

- On culture media: the size of sclerotia varied significantly ($p = 0.01$) with the isolate as well as with the type of media used. Isolate Sr. 1 produced largest sized sclerotia (mean dia: 1.375mm), Sr. 3, Sr. 4 and Sr. 5 did not differ significantly ($p = 0.01$) in respect of sclerotial size. Sclerotia produced on Sabouraud's agar were the largest (mean dia: 1.423mm) (Tab. 3), however, in size they were statistically at par ($p = 0.01$) with those produced on Asthana & Hawker's medium. Size of sclerotia was significantly ($p = 0.01$) smaller on Brown's agar (mean dia: 1.111mm) than on rest of the media. Isolates x media interactions were significant ($p = 0.01$) for the size of sclerotia. Shape of sclerotia was spherical to sub-spherical and it was similar in all the isolates on different media. The colour of sclerotia of all the isolates was white at first later turning to reddish-brown and finally to dark-brown. The type of medium neither influenced the colour nor the shape of sclerotia formed. On different culture media, maximum weight of 100 sclerotia was recorded in Sr. 1 (86.86 mg, Tab. 4) followed by Sr. 4, Sr. 2 and Sr. 3. Out of 7 media, Sabouraud's medium supported highest sclerotial weight (93.80mg) while the lowest sclerotial weight (77.6mg) was recorded on Potato Dextrose Agar.

Source : MNHN, Paris

Size - dia (mm)						
Isolates / Medium	Sr. 1	Sr. 2	Sr. 3	Sr. 4	Sr. 5	Mean of media
Potato Dextrose Agar	1.309 (.77-1.68)	1.274 (.875-1.75)	1.211 (.875-1.75)	1.221 (.875-1.575)	1.204 (.875-1.575)	1.244
Oat Meal Agar	1.384 (.875-2.1)	1.360 (.98-2.1)	1.346 (.875-1.925)	1.306 (.875-2.1)	1.285 (.91-2.1)	1.337
Brown's Agar	1.188 (.77-1.575)	1.115 (.77-1.575)	1.100 (.63-1.575)	1.047 (.77-1.575)	1.104 (.77-1.575)	1.111
Richards' Agar	1.422 (.455-2.66)	1.373 (.77-2.52)	1.099 (.595-1.75)	1.087 (.525-1.75)	1.054 (.56-1.75)	1.207
Sabouraud's Agar	1.509 (.7-2.485)	1.499 (.735-2.8)	1.273 (.63-2.73)	1.467 (.56-2.835)	1.363 (.45-2.66)	1.423
Asthana & Hawker's Agar	1.408 (.7-2.275)	1.342 (.7-2.275)	1.341 (.875-2.135)	1.363 (.805-2.135)	1.320 (.77-2.1)	1.355
Czapek's agar	1.407 (.77-2.45)	1.347 (.77-2.1)	1.376 (.77-2.275)	1.251 (.63-2.135)	1.335 (.7-2.135)	1.343

	C.D. at 5%	C.D. at 1%
Media (M)	0.052	0.068
Isolate (I)	0.044	0.058
Interaction (I x M)	0.115	0.153

Table 3: Size of the sclerotia of 5 isolates of *S. rolfsii* on different culture media. Data are mean of 4 replications (in parentheses are ranges of size).

Tableau 3: Taille des sclérotes des 5 isolats de *S. rolfsii* sur différents milieux (moyennes de 4 expériences).

Source : MNHN, Paris

Table 4: 100-sclerotia-weight of 5 isolates of *S. rolfsii* on different media.

Tableau 4: Poids de 100 sclérotes pour les 5 isolats de *S. rolfsii* sur différents milieux.

	Weight (mg)					
Isolates / Medium	Sr. 1	Sr. 2	Sr. 3	Sr. 4	Sr. 5	Mean of media
P.D.A.	85	79	75	75	74	77.6
Oat Meal Agar	85	76	78	86	75	80.6
Brown's Agar	79	87	79	88	73	81.2
Richards' Agar	90	81	78	80	79	81.6
Sabouraud's Agar	100	94	94	91	90	93.8
Asthana & Hawker's Agar	80	79	79	88	81	81.4
Czapek's Agar	89	79	88	79	78	82.6
Mean of isolate	86.86	82.14	81.57	83.85	78.57	

- On sugarbeet plant: significantly ($p = 0.01$) larger sclerotia were observed in Sr. 1 (mean dia: 1.595mm). Isolate Sr. 2 produced smallest (mean dia: 1.305mm) sclerotia (Tab. 5).

The weight of sclerotia also varied with the isolate. The sclerotia of isolate Sr. 1 had maximum weight (121mg/100 sclerotia) whereas sclerotia of Sr. 5 had minimum weight (74mg/100 sclerotia) (Tab. 5). No differences in shape and colour of sclerotia were observed amongst different isolates.

Table 5: Variation in diameter and weight of sclerotia of the 5 isolates of *S. rolfsii* formed on sugar beet cv. Ramonskaya. Data on size are mean of 4 replications (25 sclerotia per replication).

Tableau 5 - Variation du diamètre et du poids des sclérotes des 5 isolats de *S. rolfsii*, sur betterave à sucre cv. Ramonskaya (4 exp., 25 sclérotes par exp.).

Isolates	Size of sclerotia (dia in mm) Mean	Range	Weight of 100 sclerotia (mg)
Sr. 1	1.595	0.35 - 2.8	121
Sr. 2	1.305	0.49 - 2.45	113
Sr. 3	1.558	0.91 - 2.73	84
Sr. 4	1.445	0.77 - 2.45	117
Sr. 5	1.425	0.49 - 2.62	74
C.D. at 5%	0.12		
C.D. at 1%	0.17		

Pathological variability (pathogenic variations): Isolate Sr. 1 caused significantly ($p = 0.01$) higher incidences of seedling blight (97.33%) as well as root rot (91.66%) than rest of the isolates (Tab. 6). Isolates Sr. 2, Sr. 3, Sr. 4 and Sr. 5 did not exhibit significant ($p = 0.01$) difference among themselves in causing incidences of seedling blight as well as root rot.

Source : MNHN, Paris

Table 6: Pathogenic variability amongst the 5 isolates of *S. rolfsii* in causing seedling blight (mean of 5 replications) and root rot (mean of 6 replications) phases of *Sclerotium* root rot of sugar beet (in parentheses are angular values).

Tableau 6: Pathogénicité des 5 isolats de *S. rolfsii* vis-à-vis de la betterave à sucre.

Isolates	Disease incidence (%)	
	Seedling blight phase	Root rot phase
Sr. 1	97.33 (81.61)	91.66 (76.36)
Sr. 2	70.22 (56.92)	68.33 (55.89)
Sr. 3	68.44 (55.84)	70.00 (56.89)
Sr. 4	67.09 (55.05)	68.33 (55.89)
Sr. 5	67.99 (55.55)	70.00 (56.89)
C.D. at 5%	4.18	7.49
C.D. at 1%	5.70	10.13

DISCUSSION

Allison (1952), Higgins (1927) and Weber (1931) compared the morphology of isolates of *S. rolfsii* and concluded that the difference in the cultures studied were relatively small. In the present investigations, the 5 isolates were found indistinguishable in respect of colour and topography of mycelial growth. Isolate Sr. 1 displayed significantly higher radial growth and formed significantly more number of sclerotia as compared to other isolates. This indicates that isolates of the pathogen can vary in cultural characters.

In the present studies, maximum growth of all the isolates was recorded on Oat Meal Agar followed by PDA. Growth on rest of the media such as Brown's agar, Richards' agar, Sabouraud's agar, Asthana & Hawker's agar and Czapek's agar was comparatively poorer. These findings are in agreement with the results obtained by Takahashi (1927), Higgins (1927), Endo (1940) and Grover & Chona (1960). Sharma & Kaushal (1979) reported that *S. rolfsii* produced highest number of sclerotia on PDA. Similar observations were recorded in the present investigations. As observed by Indulkar (1962), Mathur & Sarbhoy (1976) and Sharma & Kaushal (1979), Richards' medium was found to be less suitable for sclerotial production.

Davey & Leach (1941) observed that sclerotia produced on sugarbeet were larger than those produced in pure culture. Similar observations were recorded in the present investigations. The size of sclerotia varied significantly with the isolates of pathogen as well as with the type of artificial medium used. The isolates x media interactions were significant for the size of sclerotia but not for number of sclerotia and radial growth. It is likely that the formation of sclerotia is more profoundly influenced by the nature and synthesis of the morphogenic factor of the fungus as reported in case of *Corticium rolfsii* by Goujon (1969, 1970) while the size of sclerotia may not be so rigidly controlled by the fungal genetic make-up. This possibility remains to be critically examined through systematically planned experiments.

Source : MNHN, Paris

Of the 5 isolates studied, one isolate (Sr. 1) differed contrastingly from the rest in radial growth and sclerotial characters. This indicates that the pathogen is highly variable physiologically.

Some investigators could not detect pathogenic variability in isolates from various sources (Taubenhaus, 1919; Weber, 1931; Allison, 1952). Epps et al. (1951) comparing pathogenicity of 4 isolates on several hosts demonstrated differences in rate of pathogenesis but no difference in total number of plants killed. Matheswaran (1979) recorded a range of variation in seedling mortality of sugarbeet cv. Ramonskaya inoculated with 9 isolates of *S. rolfsii*. In the present investigations, isolate Sr. 1 caused significantly higher incidence of seedling blight as well as root rot than rest of the isolates. This indicates that the pathogen can be variable pathogenically. The fact that the pathogen can be variable pathogenically should get due importance in programmes for resistance breeding.

Of all the isolates tested, Sr. 1 proving most aggressive displayed maximum growth on culture media. If highly aggressive isolates are always found displaying more growth on a culture medium and vice-versa then the aggressive races could be identified easily on culture medium obviating the necessity of inoculation of host plants for the purpose.

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